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SEX PHEROMONE OF THE DOGWOOD BORER, *Synanthedon scitula*

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Abstract—The sex pheromone of female dogwood borers (DWB) *Synanthedon scitula* (Harris) (Lepidoptera: Sesiidae) was determined to be an 88:6:6 ternary blend of (*Z,Z*)-3,13-octadecadienyl acetate (*Z,Z*-3,13-ODDA), (*E,Z*)-2, 13-octadecadienyl acetate (*E,Z*-2,13-ODDA), and (*Z,E*)-3,13-octadecadienyl acetate (*Z,E*-3,13-ODDA) by gas chromatographyYelectroantennographic detection (GCYEAD) and gas chromatographyYmass spectrometry (GC–MS). The major sex pheromone component, *Z,Z*-3,13-ODDA, was attractive as a single component. A blend of *Z,Z*-3,13-ODDA with 1Y3% of *E,Z*-2,13-ODDA (binary blend) was more attractive than the single component. A third component, *Z,E*-3,13-ODDA, was sometimes observed in GCYEAD analyses, and enhanced attraction to the binary blend in some field bioassays. Lures containing 1 mg of binary and ternary blends attracted 18 and 28 times more male DWB moths, respectively, than caged virgin females in field trials. Attraction was strongly antagonized by addition of as little as 0.5% of *E,Z*-3, 13-octadecadienyl acetate (*E,Z*-3,13-ODDA). In a period of 12 wk in 2004, more than 60,000 males were captured in sticky traps baited with synthetic pheromone blends in six apple orchards in Virginia, West Virginia, and North

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Carolina. Lure longevity trials showed that ~76% of the pheromone remained in rubber septum lures after 12 wk in the field.

Key Words V Dogwood borer, *Synanthedon scitula*, sex pheromone, (*Z,Z*)-3,13-octadecadienyl acetate, (*E,Z*)-2,13-octadecadienyl acetate, (*Z,E*)-3,13-octadecadienyl acetate, (*E,Z*)-3,13-octadecadienyl acetate, pheromone antagonist.

INTRODUCTION

The dogwood borer (DWB), *Synanthedon scitula* (Harris) (Lepidoptera: Sesiidae), is an important pest of at least 19 species of fruit, nut, and ornamental trees in the eastern United States and Canada (Engelhardt, 1932; Eichlin and Duckworth, 1988; Bergh and Leskey, 2003). Since the 1980s, DWB has become an increasingly important economic pest of apple (Riedl et al., 1985; Warner and Hay, 1985; Kain and Straub, 2001) largely due to the increased use of clonal, size-controlling rootstocks in high-density orchards (Marshall and Andrews, 1994). Commercially available pheromone lures for DWB rely on generalized sex pheromone components identified from other sesiid species (Tumlinson et al., 1974; Nielsen et al., 1975, 1979). This has led to inconsistencies and variability in the results from published research on DWB conducted in apple orchards, native habitats, and managed urban landscapes (reviewed in Bergh and Leskey, 2003). Furthermore, different lures marketed for monitoring DWB vary considerably in their attractiveness to and selectivity for DWB (Bergh et al., 2004). A species-specific sex pheromone identified from DWB is needed for reliable, standardized detection and monitoring of this pest.

The objectives of this study were to determine the composition of the sex pheromone of DWB and to develop a species-specific and effective pheromone lure for monitoring. Species-specific sex pheromone baited traps would be useful for comparing the seasonal life history and relative abundance of DWB in its different habitats, and accurate information on population density would facilitate management decisions in apple orchards and ornamental nurseries. The failure of previous attempts to manage DWB using mating disruption (Pfeiffer and Killian, 1999) may have been due, in part, to an inadequate characterization of its sex pheromone. Mating disruption products for the congeneric peach tree borer, *Synanthedon exitiosa* (Say), and lesser peach tree borer, *S. pictipes* (Grote and Robinson), are effective (Gentry and Snow, 1984; Pfeiffer et al., 1991; Agnello and Kain, 2002) and commercially available, and identification of the DWB sex pheromone may enable development of this management approach for DWB as well. It will also enable the investigation of pheromone-based, premating reproductive isolation mechanisms among several sympatric sesiid congeners. This article describes the determination of the sex pheromone composition of the DWB, and field trapping studies that examined and compared the responses of males to different geometric isomers, blend ratios, pheromone antagonists, and virgin females. The release rates and longevities of binary and ternary pheromone lures were quantified under field conditions.

METHODS AND MATERIALS

Insects. Larvae (~100) of DWB were excavated from burrknot tissue of apple trees (Jefferson and Berkeley Counties, WV, USA and Frederick County, VA, USA) with obvious signs of infestation, including frass and entry wounds, in October and November of 2002 and 2003. Larvae were brought to the laboratory and reared on general-purpose lepidopteran diet (Bioserv, Frenchtown, NJ, USA) in an incubator at 25°C (16L:8D) until pupation. Pupae were sexed according to the characteristics described by Leskey and Bergh (2003) and held individually in 1-oz clear plastic cups (Jet Plastica Industries, Hatfield, PA, USA) with a small piece of moistened cotton dental wick and topped with plastic caps. Pupae were sent to Beltsville, MD, USA in January 2002 and 2003. Upon arrival, the pupae were kept in an insectary at 25°C and 16L:8D photoperiod until adult emergence. Absorbent cotton moistened with 8% sugar water was provided as a food source for emerged moths. Female moths were transferred to an effluvial collection device for collection of volatiles.

Effluvial Collections. Volatiles were collected using six groups of 1- to 13-d-old virgin females (3Y10 females per group) at room temperature and 16L:8D photoperiod. The moths were introduced separately into three 1-l, four-necked glass containers (Zhang et al., 1994). Air was drawn into the container through 6- to 14-mesh activated charcoal (Fisher Scientific, Pittsburgh, PA, USA) and out of the container through two traps (15 cm × 1.5-cm o.d.) containing Super Q (200 mg each; Alltech Associates, Inc., Deerfield, IL, USA) by vacuum (~1 l/min). Female moths were fed a 10% sugar solution on cotton balls and aerated continuously for 3Y7 d at room temperature and 16L:8D photoperiod. The adsorbent traps were changed every 48 hr. Adsorbents were eluted with methylene chloride (4 × 0.5 ml, spectrometry grade, EMD Chemicals Inc., Gibbstown, NJ, USA) and the eluates (2 ml/sample) were concentrated to ~20 21 under a nitrogen stream and stored at −30°C until analysis.

Pheromone Gland Extractions. Pheromone gland extracts were obtained during photophase from the six groups of 2- to 13-d-old virgin females that had been used in aerations. A female abdomen was compressed gently until the ovipositor everted from the abdominal tip. The ovipositor was then excised with microscissors into a conical glass vial containing ~100 21 methylene chlorideYmethanol (3:1), and the glands were soaked for at least 2 hr at room temperature. The extracts were removed, and the glands were reextracted with 100 21 methylene chlorideYmethanol (Zhang and Polavarapu, 2004). The combined solution was concentrated to ~20 2l under a nitrogen stream and stored at −30°C until analysis.

Electrophysiological Recordings and Mass Spectrometry. The coupled gas chromatographyYelectroantennographic detection (GCYEAD) system used was as previously described (Zhang et al., 1997; Zhang and Polavarapu, 2003). For GCYEAD analysis, an Hewlett-Packard (HP) 6890 gas chromatograph equipped with a 60 m × 0.25-mm i.d., 0.25-2m film-thickness DB-WAXETR capillary column (J&W Scientific Inc., Folsom, CA, USA; 120°C for 2 min, 10°C/min to 250°C, hold for 10 min) or a 60 m × 0.25-mm i.d., 0.25-2m film-thickness DB-5 capillary column (J&W Scientific Inc., 100°C for 2 min, 15°C/min to 250°C, hold for 10 min) in splitless mode with hydrogen carrier gas (1.4 ml/min) was used.

Gas chromatographyYmass spectrometry (GCYMS) was conducted on an HP 6890 GC coupled to an HP 5973 mass-selective detector using an identical DB-WAXETR capillary column (120°C for 2 min, 15°C/min to 230°C, hold for 25 min), but with helium as carrier gas. A 70-eV electron beam was used for sample ionization.

Chemicals. (*Z,Z*)-3,13-Octadecadienyl acetate (~90% purity, Bedoukian Research, Danbury, CT, USA) was purified by flash chromatography using 15% $AgNO_3$ on silica gel 60 (230Y400 mesh, EM Science, Gibbstown, NJ, USA), eluted with 2:10 CH_2Cl_2:hexane. The fractions with <0.3% of the impurity, *E, Z*-3,13-ODDA, were combined. All other pheromone standards were purchased from PheroBank (Wageningen, the Netherlands), and purities of chemicals were checked on a 60-m polar DB-WAXETR GC capillary column before preparing lures for field studies (>97% purity).

Field Trapping Tests. Red natural rubber septa (5 mm, Wheaton, Millville, NJ, USA) loaded with the desired rates of *Z,Z*-3,13-ODDA and the blends in ~20 2l of hexane solution were used for field trials with hexane controls. The same amount of solvent (hexane) was loaded on the septum for the blank control. After loading, the solvent was allowed to evaporate in a fume hood for 30 min. Lures were then wrapped in aluminum foil, stored in 20-ml plastic vials and shipped by express carrier on the same day. Upon arrival, the lures were kept in a freezer at −10°C until deployed.

All field tests were conducted in commercial apple orchards in West Virginia, Virginia, and North Carolina using Pherocon 1C (North Carolina and West Virginia) or Delta sticky traps (Virginia) (Trécé, Salinas, CA, USA). Traps were placed in trees at ~1.2 m above the ground (Riedl et al., 1985). At each location, experimental lures were randomized within each of three to five rows (depending on the number of replicates per test) that were separated by at least one buffer row, and traps were spaced at a minimum of ~20-m intervals within a row. Traps were rotated among positions within each row at weekly intervals for the duration of each test. The number of dogwood borer and other male sesiid moths captured were recorded weekly.

In the first comparison, the following lures were deployed: the single component (100% *Z,Z*-3,13-ODDA), the binary blend (*Z,Z*-3,13-ODDA/*E,Z*-2,13-ODDA = 94:6), a second two-component blend (*Z,Z*-3,13-ODDA/*Z,E*-3,13-ODDA = 94:6), the ternary blend (*Z,Z*-3,13-ODDA/*E,Z*-2,13-ODDA/*Z,E*-3,13-ODDA = 88:6:6), and the control. To evaluate the effect on trap catch of addition of a third component, 0.5, 1, 3, or 10% of *Z,E*-3,13-ODDA was added to the base binary blend. Similarly, 0.5, 1, 3, or 10% of *E,Z*-2,13-ODDA was added to a blend consisting of *Z,Z*-3,13-ODDA/*Z,E*-3,13-ODDA (94:6) to evaluate the addition of *E,Z*-2,13-ODDA on trap capture. In another comparison, 0.5, 1, 2, or 3% *E,Z*-3,13-ODDA was added to the ternary blend. Caged virgin females (2- to 4-d-old and one female per cage) were deployed in traps to provide a natural source of pheromone for comparison with traps baited with the binary and ternary blends over a 3-wk period.

Lure Analyses. The binary and ternary lures exposed in the field during 6, 8, and 12 wk were collected and placed individually into 20 ml hexane in a 25-ml vial and soaked for 48 hr. GC analyses of pheromone lures were conducted with an HP 6890 GC equipped with a DB-5 capillary column by injecting 1 2l of each extract. Pure *Z,Z*-3,13-ODDA was used as the standard, and remaining pheromone concentrations were obtained by comparison with this standard analyzed under the same conditions.

Statistics. Data from control traps were omitted from the statistical analyses because of zero trap catches. Unless otherwise specified, trap catch data from each test were log transformed (log $X + 1$) to normalize the variance before analysis. Means were compared by one-way analysis of variance (ANOVA) followed by RyanYEinotYGabrielYWelsch range test (SPSS 10.0 for Windows; George and Mallery, 2002) for significance at $\alpha = 0.05$.

RESULTS

Analysis of Sex Pheromone Components in Female Effluvial Collections and Gland Extracts. A typical coupled GCYEAD graph is shown in Figure 1, exhibiting two EAD responses in female gland extract (Figure 1A) and three EAD responses in effluvial extract (Figure 1B). The mass spectrum of the component in the gland extracts that elicited the largest EAD response (peak 1) exhibited the highest mass ion at *m*/*z* 308 (1) along with a comparatively strong mass fragment at *m*/*z* 248 (22) and other fragments: *m*/*z* 219 (6), 149 (14), 135 (27), 121 (24), 109 (37), 96 (66), 81 (85), 67 (80), and 55 (81). It closely matched the spectra of authentic 3,13-ODDA and 2,13-ODDA. Among the EAD responses, peaks 1 and 2 coincided with *Z,Z*-3,13-ODDA and *E,Z*-2,13-ODDA, respectively, in GC retention times on both capillary columns (Table 1), suggesting that *Z,Z*-3,13-ODDA and *E,Z*-2,13-ODDA were likely candidates for

TABLE 1. RETENTION TIMES OF EAD-ACTIVE COMPOUNDS OBTAINED FROM GLAND AND EFFLUVIAL EXTRACTS OF FEMALE DWB AND SYNTHETIC 3,13- AND 2,13-OCTADECADIENYL ACETATES

Compounds	Retention time (min) DB-WAXETR	DB-5	EAD response to 10 ng dose (mV, mean ± SD)[a]
From females			
(*Z,Z*)-3,13-Octadecadienyl acetate	14.48	13.82	
(*E,Z*)-2,13-Octadecadienyl acetate	14.75	13.93	
Synthetic			
(*E,E*)-3,13-Octadecadienyl acetate	14.39	13.81	0.13 ± 0.03 ab
(*Z,E*)-3,13-Octadecadienyl acetate	14.42	13.80	0.24 ± 0.04 a
(*E,Z*)-3,13-Octadecadienyl acetate	14.44	13.83	0.30 ± 0.09 a
(*Z,Z*)-3,13-Octadecadienyl acetate	14.48	13.82	0.30 ± 0.10 a
(*Z,E*)-2,13-Octadecadienyl acetate	14.46	13.84	0.03 ± 0.03 b
(*Z,Z*)-2,13-Octadecadienyl acetate	14.53	13.86	0.05 ± 0.04 b
(*E,E*)-2,13-Octadecadienyl acetate	14.68	13.90	0.04 ± 0.02 b
(*E,Z*)-2,13-Octadecadienyl acetate	14.75	13.93	0.20 ± 0.13 ab

[a]Means followed by different letters are significantly different at $\alpha = 0.05$ ($N = 3$, $df = 7.16$, $F = 8.30$, $P < 0.001$).

Z,Z-3,13-ODDA and *E,Z*-2,13-ODDA, and with a ratio of 94:6. The identity of the second component eliciting an EAD response (peak 2) was confirmed by co-injection with a synthetic standard of *E,Z*-2,13-ODDA in GCYMS. Authentic standards of synthetic *Z,Z*-3,13-ODDA and *E,Z*-2,13-ODDA also elicited strong antennal responses (Figure 1C). However, the third component of the effluvial extract that sometimes elicited EAD responses was not detected in gland extracts via selected ion monitoring. GCYEAD analyses of the four synthetic geometric isomers of 3,13-ODDA revealed that all elicited significant EAD responses (Table 1) and that the retention times of *Z,E*-3,13-ODDA were identical to those of peak 3 on both capillary columns. Therefore, the minor component in the effluvial extract (peak 3) that elicited antennal responses was tentatively determined to be *Z,E*-3,13-ODDA, and the ternary blend ratio was estimated to be 88:6:6 (*Z,Z*-3,13-ODDA/*E,Z*-2,13-ODDA/*Z,E*-3,13-ODDA).

Field Trapping Tests. The numbers of male moths caught in initial field studies by using traps baited with septa containing 1 mg of the single major component, two 2-component blends, and the ternary blend are summarized in Figure 2. Catches of male moths in orchards in three states all showed that the major sex pheromone component, *Z,Z*-3,13-ODDA, was attractive as a single component. The binary blend of *Z,Z*-3,13-ODDA with 6% *E,Z*-2,13-ODDA was more attractive than the single component. Addition of the second minor component, *Z,E*-3,13-ODDA, to create the ternary blend, further enhanced attraction (Figure 2).

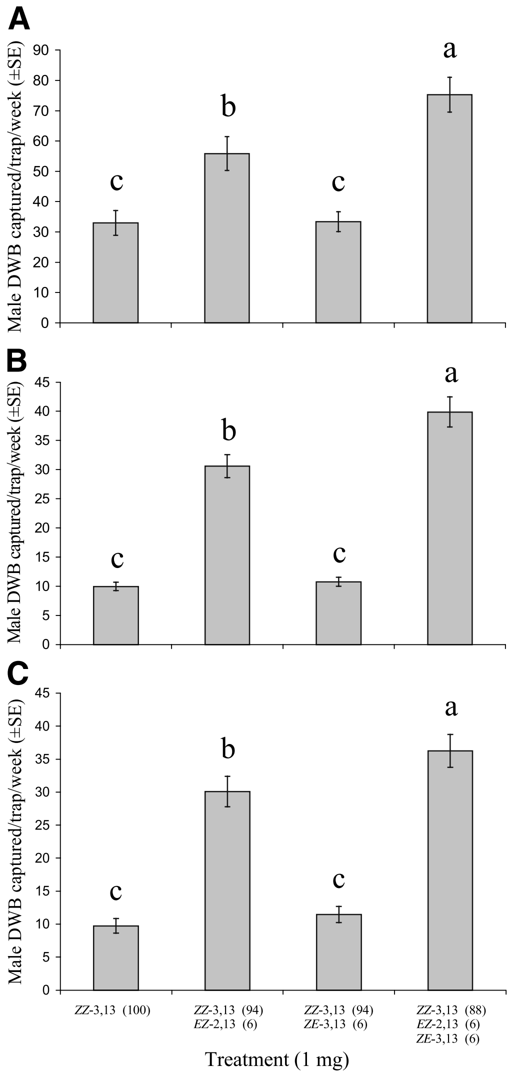

FIG. 2. Male DWB captured in traps with different DWB pheromone blends conducted (A) from May 18 to July 8, 2004, in North Carolina (total number of male DWBs trapped was 14,101, $N = 70$, $df = 3{,}276$, $F = 63.3$), (B) from June 2 to August 25, 2004, in Virginia (total number of male DWBs trapped was 11,917, $N = 130$, $df = 3{,}516$, $F = 112.1$), and (C) from June 1 to August 23, 2004, in West Virginia (total number of male DWBs trapped was 10,506, $N = 120$, $df = 3{,}476$, $F = 223.0$). Bars superscripted by different letters are different (arcsin $\sqrt{p}$ transformed, where p is the original proportion; $P < 0.05$).

However, in a follow-up trial, addition of 0.5 to 10% of *Z,E*-3,13-ODDA to the binary blend of *Z,Z*-3,13-ODDA and *E,Z*-2,13-ODDA (94:6) had no effect (Figure 3). In contrast, when 0.5 to 10% of *E,Z*-2,13-ODDA was added to a binary blend of *Z,Z*-3,13-ODDA and *Z,E*-3,13-ODDA (94:6), higher numbers of males were attracted to most of the ternary blends compared to the binary blend (Figure 4). In a subsequent test, the addition of as little as 0.5% of the geometric isomer, *E,Z*-3,13-ODDA, to the most attractive ternary blend (*Z,Z*-3,13-ODDA/*E,Z*-2,13-ODDA/*Z,E*-3,13-ODDA = 88:6:6) strongly antagonized the attraction of male DWB to traps (Figure 5).

In the final field test, we compared the attractiveness of binary and ternary blends to virgin females. Rubber septa loaded with 1 mg doses of binary and ternary blends attracted more males than traps baited with one virgin female (Figure 6).

Our pheromone blends showed greatly improved species specificity in comparison to results reported from the use of commercially available lures (Rogers and Grant, 1990; Davidson et al., 1992; Braxton and Raupp, 1995; Pfeiffer and Killian, 1999; Bergh et al., 2004). In the most recent comparison of commercially available lures, the Scenturion dogwood borer lure was the most attractive and species specific, with the percentage of DWB captured ranging

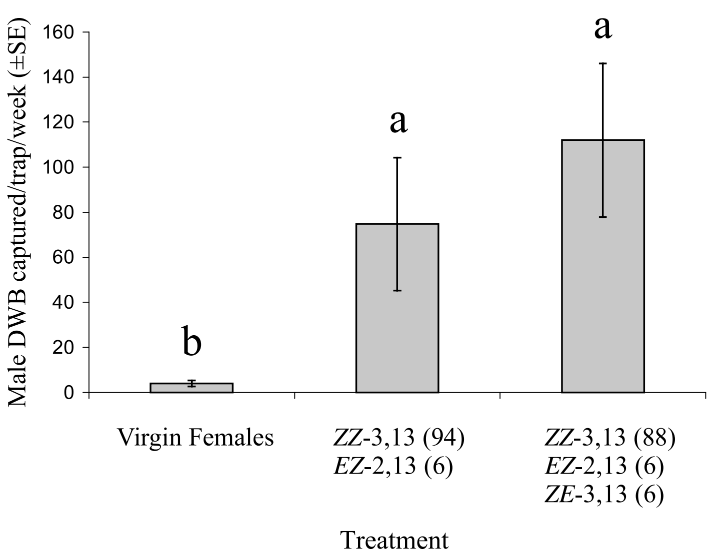

FIG. 6. Male DWB captured in traps baited with synthetic pheromone or virgin females from August 16 to September 13, 2004, in West Virginia (total number of male DWB trapped was 763, $N = 4$, $df = 2,9$, $F = 25.72$). Bars superscripted by different letters are different ($P < 0.05$).

TABLE 2. MEAN AMOUNT OF PHEROMONE COMPONENTS REMAINING IN LURES AFTER EXPOSURE UNDER FIELD CONDITIONS[a]

		Amount remaining (mg)/septum (mean ± SD in %)		
Orchard	Blend	6 wk[b]	8 wk[c]	12 wk[d]
1	Binary	0.87 ± 5.5		
2	Binary		0.79 ± 7.9	
1	Ternary	0.86 ± 9.4		
2	Ternary		0.84 ± 6.2	
3	Binary			0.72 ± 3.8
4	Binary			0.75 ± 1.7
3	Ternary			0.81 ± 2.6
4	Ternary			0.77 ± 5.1

[a] Original dose was 1.0 mg/septum. Values listed represent sum of remaining components.
[b] Exposed from May 18 to June 29, 2004, in North Carolina.
[c] Exposed from May 18 to July 9, 2004, in North Carolina.
[d] Exposed from June 2 to August 25, 2004, in Virginia.

from 33 to 86% of all Sesiidae captured (Bergh et al., 2004). In contrast, about 92% of all moths captured with our single-component or two-component blend (*Z,Z*-3,13-ODDA/*Z,E*-3,13-ODDA = 94:6) were DWB males. Even greater levels of specificity were observed with our binary (*Z,Z*-3,13-ODDA/*E,Z*-2,13-ODDA = 94:6) and ternary blends (*Z,Z*-3,13-ODDA/*E,Z*-2,13-ODDA/*Z,E*-3,13-ODDA = 88:6:6), with which over 97% of moths caught were DWB males (Leskey et al., 2005).

Lure longevity and release rate were evaluated using lures loaded with 1 mg of the binary and ternary blends and exposed in the field for 6, 8, and 12 wk. GC analyses indicated that 87% of the pheromone remained in the rubber septum lures exposed for 6 wk in the field (May 18 to June 29, 2004) in North Carolina, 82% of the pheromone remained in lures exposed for 8 wk (8 May to July 9, 2004) in Virginia, and 76% of the pheromone remained in lures exposed for 12 wk (June 2 to August 25, 2004) in Virginia (Table 2).

DISCUSSION

Following the discovery of *E,Z*- and *Z,Z*-3,13-ODDA as components of the sex pheromones of lesser peach tree borer and peach tree borer, respectively (Tumlinson et al., 1974), the geometrical isomers and corresponding alcohols of 3,13-ODDA have been reported as the main pheromone components of many sesiid species (Nielsen et al., 1979; Tumlinson, 1979; Snow et al., 1985). A positional isomer, *E,Z*-2,13-ODDA, was later identified as a component of the sex pheromone of other Sesiidae, including grape root borer, *Vitacea* *polistiformis* (Harris), maple clearwing, *S. acerrubri* (Engelhardt), and squash vine borer, *Melittia satyriniformis* (Hübner) (Schwarz et al., 1983; Snow et al., 1987).

Z,Z-3,13-ODDA was reported as a sex attractant for DWB, based on a combination of electroantennogram tests and the results of field trapping tests in which many compounds were screened (Nielsen et al., 1975, 1979). However, specific identification of the DWB sex pheromone has not been reported previously. Based on GCYEAD analyses of effluvial collections, gland extracts, and synthetic isomers of 3,13- and 2,13-ODDA, and field trapping tests, we have confirmed that *Z,Z*-3,13-ODDA is the main pheromone component of DWB. As a single component, it attracts male DWB, in concurrence with the results of Nielsen et al. (1975). However, we also showed that two other components increased attraction of male DWB to Z,Z-3,13-ODDA. When *Z,Z*,-3,13-ODDA was combined with *E,Z*-2,13-ODDA in a 94:6 blend (the natural ratio), moth captures were greater than with *Z,Z*-3,13-ODDA alone or with the ternary blend of *Z,Z*-3,13-ODDA in combination with *Z,E*-3,13-ODDA (94:6). Our findings explain the results reported in previous studies, in which DWB moths were captured in traps used for evaluating seasonal flight activity of grape root borer, which contained lures with a blend of 99:1 (*E,Z*-2,13-ODDA/*Z,Z*-3,13-ODDA) (Alm et al., 1989; Snow et al., 1991). The ternary blend (*Z,Z*-3,13-ODDA/*E,Z*-2,13-ODDA/*Z,E*-3,13-ODDA = 88:6:6) proved to be more attractive than any other treatment across all locations in one trial (Figure 2), but in other trials (Figures 3 and 6), it was no better than the 94:6 binary blend of *Z,Z*-3,13-ODDA:*E,Z*-2,13-ODDA.

Thus, two pheromone components, *Z,Z*-3,13-ODDA and *E,Z*-2,13-ODDA, appear to be essential for maximal attraction of male DWB. When as little as 1% *E,Z*-2,13-ODDA was added to a 94:6 (*Z,Z*-3,13-ODDA/*Z,E*-3,13-ODDA) blend, captures increased at all locations (Figure 4). However, we did not observe a similar response when *Z,E*-3,13-ODDA was added to a 94:6 (*Z,Z*-3,13-ODDA/*E,Z*-2,13-ODDA) blend (Figure 3). We believe that *Z,E*-3,13-ODDA may indeed synergize the response to the binary blend (*Z,Z*-3,13-ODDA/*E,Z*-2,13-ODDA) based on the finding that in one set of trials, more male moths were caught at all locations with this ternary blend than with the binary blend (Figure 2). However, male DWB may not have been able to discriminate among treatments in our trial that evaluated the addition of 0.5Y10% *Z,E*-3,13-ODDA to the binary blend (*Z,Z*-3,13-ODDA/*E,Z*-2,13-ODDA) (Figure 3), because the distances between stimuli were approximately 20 m, whereas in our other field trial that compared the binary and ternary blends with our other treatments, the distance between these two attractive stimuli was as much as 100 m.

E,Z-3,13-ODDA, the major component of lesser peach tree borer sex pheromone (Tumlinson et al., 1974), has been described as a potential antagonist to the attraction of male DWB (Karandinos et al., 1977; Greenfield, 1978; Warner and Hay, 1985), whereas Snow et al. (1985) concluded that it had no effect on DWB response to *Z,Z*-3,13-ODDA. Here, we document the strongly antagonistic effect of *E,Z*-3,13-ODDA on the response of male DWB to its sex pheromone. In field trials, as little as 0.5% *E,Z*-3,13-ODDA added to our ternary blend strongly reduced trap captures at all locations (Figure 5). The importance of this particular compound in terms of niche separation is clear. In EAG studies, male DWB antennae responded as strongly to *E,Z*-3,13-ODDA as to *Z,Z*-3,13-ODDA (Nielsen et al., 1979). Interestingly, when *Z,Z*-3,13-ODDA, which is also a component of the peach tree borer sex pheromone, was added to *E,Z*-3,13-ODDA, the pheromone of lesser peach tree borer, addition of as little as 0.5% of *Z,Z*-3,13-ODDA completely antagonized the response of lesser peach tree borer males (Tumlinson et al., 1974; Karandinos et al., 1977; Mclaughlin et al., 1977). Understanding the role of attractants, antagonists, and synergists among isomers of 3,13-ODDA and 2,13-ODDA will aid in understanding mechanisms of reproductive isolation among sympatric, congeneric sesiid species.

Measurement of the amount of pheromone remaining in field-aged lures showed that the compounds that compose the DWB sex pheromone are released slowly from red rubber septa, with most of a 1-mg applied dose being recovered after 12 wk of field exposure (Table 2). The experiment was terminated only because of the seasonal decline in DWB populations, rather than due to a decline in the lure attractiveness, and we believe that lures formulated with 1 mg of pheromone or less (Leskey et al., 2005) will remain effective for the duration of the annual period of emergence and flight activity of DWB.

In summary, our synthetic lures were extremely attractive to male DWB. Traps baited with the ternary blend containing *Z,Z*-3,13-ODDA, *E,Z*-2,13-ODDA, and *Z,E*-3,13-ODDA (88:6:6) captured ~28 times more male DWB than traps baited with caged, virgin female DWB. The identification of the DWB pheromone represents a major advance in several respects. First, a reliable and standardized pheromone-based monitoring system can now be delivered to growers, providing them with the means to determine seasonal flight activity and infestation levels in orchards and nurseries, and enabling more informed management decisions. Second, the use of an improved, species-specific blend of compounds should advance our ability to investigate the effectiveness of mating disruption and attract-and-kill management tactics for this pest. Third, the behavioral antagonism shown by the addition of *E,Z*-3,13-ODDA to the ternary blend suggests strong reproductive isolation barriers between sympatric sesiid species. Characterization of the DWB sex pheromone will enable further investigation of premating mechanisms of reproductive isolation among DWB, peach tree borer, and lesser peach tree borer, based on their different behavioral responses to isomers of ODDA.

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REFERENCES

AGNELLO, A. M. and KAIN, D. P. 2002. Evaluation of pheromone disruption in combination with insecticide application for control of peachtree borers. *N. Y. Fruit Q.* 10:29Y31.

ALM, S. R., WILLIAMS, R. N., PAVUK, D. M., SNOW, J. W., and HEINLEIN, M. A. 1989. Distribution and seasonal flight activity of male grape root borers (Lepidoptera: Sesiidae) in Ohio. *J. Econ. Entomol.* 82:1604Y1608.

BERGH, J. C. and LESKEY, T. C. 2003. Biology, ecology, and management of dogwood borer in eastern apple orchards. *Can. Entomol.* 135:615Y635.

BERGH, J. C., LESKEY, T. C., and ZHANG, A. 2004. Discrimination by male dogwood borer, *Synanthedon scitula* (Lepidoptera: Sesiidae), among traps baited with commercially available pheromone lures. *J. Econ. Entomol.* 97:344Y352.

BRAXTON, S. M. and RAUPP, M. J. 1995. An annotated checklist of clearwing borer pests of ornamental plants trapped using commercially available pheromone lures. *J. Arbor.* 21: 177Y180.

DAVIDSON, J. A., GILL, S. A., and RAUPP, M. J. 1992. Controlling clearwing moths with entomopathogenic nematodes: the dogwood borer case study. *J. Arbor.* 18:81Y84.

EICHLIN, T. D. and DUCKWORTH, W. D. 1988. Sesioidea: Sesiidae, pp. 1Y176, *in* B. Dominick (ed.). The Moths of America North of Mexico. Fascicle 5.1. Wedge Entomological Research Foundation, Washington, DC.

ENGELHARDT, G. P. 1932. Business proceedings of the eastern branch of the American Association of Economic Entomologists. *J. Econ. Entomol.* 25:239Y294.

GENTRY, C. R. and SNOW, J. W. 1984. Disruption of mating by male lesser peachtree borers and peachtree borers in a pheromone permeated peach orchard. *J. Ga. Entomol. Soc.* 19:350Y356.

GEORGE, D. and MALLERY, P. 2002. SPSS for Windows Step by Step: a Simple Guide and Reference 4th edn. Allyn & Bacon, Boston.

GREENFIELD, M. D. 1978. Niche segregation of adult clearwing moths (Lepidoptera: Sesiidae) in Wisconsin, p. 38. Ph.D. thesis. University of Wisconsin, Madison.

KAIN, D. and STRAUB, R. W. 2001. Status of borers infesting apple burr knots and their management in New York orchards. *N. Y. Fruit Q.* 9:10Y12.

KARANDINOS, M. G., TUMLINSON, J. H., and EICHLIN, T. D. 1977. Field evidence of synergism and inhibition of the Sesiidae sex pheromone system. *J. Chem. Ecol.* 3:57Y64.

LESKEY, T. C. and BERGH, J. C. 2003. A simple character for sex differentiation of pupae and pupal exuviae of the dogwood borer (Lepidoptera: Sesiidae). *Fla. Entomol.* 86:379Y381.

LESKEY, T. C., BERGH, J. C., WALGENBACH, J. F., and ZHANG, A. 2005. Improved attractiveness and specificity of pheromone-baited traps for male dogwood borer, *Synanthedon scitula* Harris (Lepidoptera: Sesiidae). Environ. Entomol. in press.

MARSHALL, D. W. and ANDREWS, P. K. 1994. Trends in Washington State's apple industry. *HortTechnology* 4:1Y16.

MCLAUGHLIN, J. R., TUMLINSON, J. H., and SHARP, J. L. 1977. Absence of synergism in the response of Florida lesser peachtree borer males to synthetic sex pheromone. *Fla. Entomol.* 60:27Y29.

NIELSEN, D. G., PURRINGTON, F. F., TUMLINSON, J. H., DOOLITTLE, R. E., and YONCE, C. E. 1975. Response of male clearwing moths to caged virgin females, female extracts, and synthetic sex attractants. *Environ. Entomol.* 4:451Y454.

NIELSEN, D. G., PURRINGTON, F. F., and SHAMBAUGH, G. F. 1979. EAG and field responses of sesiid males to sex pheromones and related compounds, pp. 11Y26, *in* J. W. Neal (ed.). Pheromones of the Sesiidae. SEA, U.S. Dept. Agric. ARR-NE-6, Washington, DC.

PFEIFFER, D. G. and KILLIAN, J. C. 1999. Dogwood borer (Lepidoptera: Sesiidae) flight activity and an attempt to control damage in 'Gala' apples using mating disruption. *J. Entomol. Sci.* 34: 210Y218.

PFEIFFER, D. G., KILLIAN, J. C., RAJOTTE, E. G., HULL, L. A., and SNOW, J. W. 1991. Mating disruption for reduction of damage by lesser peachtree borer (Lepidoptera: Sesiidae) in Virginia and Pennsylvania peach orchards. *J. Econ. Entomol.* 84:218Y223.

RIEDL, H., WEIRES, R. W., SEAMAN, A., and HOYING, S. A. 1985. Seasonal biology and control of the dogwood borer, *Synanthedon scitula* (Lepidoptera: Sesiidae) on clonal apple rootstocks in New York. *Can. Entomol.* 117:1367Y1377.

ROGERS, L. E. and GRANT, J. F. 1990. Infestation levels of dogwood borer (Lepidoptera: Sesiidae) larvae on dogwood trees in selected habitats in Tennessee. *J. Entomol. Sci.* 25:481Y485.

SCHWARZ, M., KLUN, J. A., LEONHARDT, B. A., and JOHNSON, D. T. 1983. (*E,Z*)-2,13-Octadecadien-1-ol acetate. A new pheromone structure for sesiid moths. *Tetrahedron Lett.* 24:1007Y1010.

SNOW, J. W., EICHLIN, T. D., and TUMLINSON, J. H. 1985. Seasonal captures of clearwing moths (Sesiidae) in traps baited with various formulations of 3,13-octadecadienyl acetate and alcohol. *J. Agric. Entomol.* 2:73Y84.

SNOW, J. W., SCHWARZ M., and KLUN J. A. 1987. The attraction of the grape root borer, *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae) to (*E,Z*)-2,13-octadecadienyl acetate and the effects of related isomers on attraction. *J. Entomol.* 371Y374.

SNOW, J. W., JOHNSON, D. T., and MEYER, W. L. 1991. The seasonal occurrence of the grape root borer, (Lepidoptera: Sesiidae) in the eastern United States. *J. Entomol. Sci.* 26:157Y168.

TUMLINSON, J. H. 1979. The chemistry of Sesiidae pheromones, pp. 1Y10, *in* W. Neal (ed.). Pheromones of the Sesiidae. SEA U.S. Dept. Agric. ARR-NE-6, Washington, DC.

TUMLINSON, J. H., YONCE, C. E., DOOLITTLE, R. E., HEALTH, R. R., GENTRY, C. R., and MITCHELL, E. R. 1974. Sex pheromones and reproductive isolation of the lesser peachtree borer and peachtree borer. *Science (Washington, D. C.)* 185:614Y616.

WARNER, J. and HAY, S. 1985. Observations, monitoring, and control of clearwing borer (Lepidoptera: Sesiidae) on apple in central Ontario. *Can. Entomol.* 117:1471Y1478.

ZHANG, A. and POLAVARAPU, S. 2003. Sex pheromone of the cranberry blossom worm, *Epiglaea apiata*. *J. Chem. Ecol.* 29:2153Y2164.

ZHANG, A. and POLAVARAPU, S. 2004. Identification of a sex pheromone component for the blueberry leafminer *Caloptilia porphyretica*. *J. Chem. Ecol.* 30:1531Y1545.

ZHANG, A., FACUNDO, H. T., ROBBINS, P. S., LINN, C. E. JR, HANULA, J. L., VILLANI, M. G., and ROELOFS, W. L. 1994. Identification and synthesis of female sex pheromone of Oriental beetle, *Anomala orientalis* (Coleoptera: Scarabaeidae). *J. Chem. Ecol.* 20:2415Y2427.

ZHANG, A., ROBBINS, P. S., LEAL, W. S., LINN, C. E. JR, VILLANI, M. G., and ROELOFS, W. L. 1997. Essential amino acid methyl esters: major sex pheromone components of the cranberry white grub *Phyllophaga anxia* (Coleoptera: Scarabaeidae). *J. Chem. Ecol.* 23:231Y245.